## REMARKS

Claims 1-13 and 29-45 were previously canceled from the case. Claims 14, 19, 20, 23, and 25 have been amended herein. Claims 14-28 remain in the case. Favorable reconsideration is respectfully requested.

The amendment to Claims 14 and 23 finds support in the specification at page 10, second full paragraph. Dependent Claims 19, 20, and 25 have been amended to maintain consistency with the amendments made to their corresponding base claims. No new matter is added.

A Revocation of Power of Attorney, Granting of Power of Attorney, and Change of Address accompanies this paper, as does a Certificate Under 37 CFR §3.73(b). The Rule 3.73(b) Certificate establishes the Wisconsin Alumni Research Foundation as the owner by assignment of the present application. Please forward all further communications in this matter to:

Intellectual Property Dept.
DeWitt Ross & Stevens S.C.
US Bank Building
8000 Excelsior Drive, Suite 401,
Madison, WI 53717-1914.

The following remarks address the issues presented in the Office Action in the order of their appearance.

**Objection to Claim 23 and Rejection of Claim 23 under 35 U.S.C. §112, Second Paragraph:**

Applicants submit that both the objection to Claim 23 and the rejection of Claim 23 under §112, second paragraph, have been overcome by an appropriate amendment to the claim. Specifically, clause (a), sub-clause (ii) has been amended to refer back to "trehalose" rather than the "polyhydroxy compound."

Withdrawal of the objection and the rejection are respectfully requested.

**Rejection of Claims 14, 16, 17, and 19-21 Under 35 U.S.C. §102(b) in View of Ahmad et al. (1997) *Transplantation Proceedings* 29:355-356:**

As applied to Claims 14, 16, 17, and 21, this rejection is believed to have been overcome by appropriate amendment to Claim 14. Specifically, the word "about" has been removed from the lower range of the total amount by weight of the polyhydroxy compound recited on Claim 14, step (a)(ii). Specifically, Claim 14 as amended now positively requires, in step (a)(ii), an aqueous preservation medium comprising a polyhydroxy compound in an amount of "from ~~about~~ 5% to about 60% by weight by weight of the medium." The Ahmad et al. paper neither discloses nor suggests an aqueous preservation medium containing this amount of polyhydroxy compound. This can be confirmed by analyzing the values presented in Table 1 of the Ahmed et al. reference.

Table 1 of Ahmed describes two solutions: a first solution designated "PBS140" and a second solution designated "HOC." The "HOC" solution **is not** relevant to the present claims because the "HOC" solution **does not** contain any phosphate ions. See the right-hand column of Table 1 of the Ahmad et al. paper.

The "PBS140" solution of Ahmad et al. contains 69 mMol phosphate (in the form of $Na_3PO_4$, *i.e.*., "sodium phosphate," see the first paragraph of the Ahmad et al. paper) and 140 mMol sucrose. Thus, the "PBS140" solution described in the middle column of Table 1 of Ahmad et al. has the following composition on a percent-by-weight basis:

1000 mL $H_2O$ = 1000 g $H_2O$.

0.140 moles sucrose x (342.3 g sucrose/mole of sucrose) = 47.92 g sucrose.

0.069 moles $PO_4$ (in the form of $Na_3PO_4$) x (163.9 g $Na_3PO_4$/mole of $Na_3PO_4$) = 11.31 g $Na_3PO_4$.

The total weight of the solution is therefore:

1000 g + 47.92 g sucrose + 11.31 g $Na_3PO_4$ = 1,059.23 g.

And the percent by weight sucrose in the solution is therefore:

47.92 g sucrose ÷1,059.23 g total weight x 100% = **4.52% by wt. sucrose**.

In short, the Ahmad et al. paper **does not** disclose a solution comprising both phosphate ions and a polyhydroxy compound (sucrose in the case of the Ahmad et al. paper) wherein the

polyhydroxy compound is present in an amount ranging from "5% by weight to about 60% by weight," as is positively required by the language of Claim 14.

Applicants thus submit that as applied to Claims 14, 16, 17, and 21, this rejection has been overcome.

As applied to Claims 19 and 20, this rejection is respectfully traversed. The Ahmad et al. solution simply does not contain anywhere near 10% by weight sucrose. Claims 19 and 20 positively require that the medium used in the method contain a polyhydroxy compound in an amount ranging "from about 10% to about 30% by weight of the medium." The Ahmad et al. paper in no way discloses such a solution. As indicated by the above calculation, the "PBS140" solution described in Table 1 of Ahmad et al. contains only 4.52% by weight of a polyhydroxy compound, namely sucrose. The phrase "**about** 10% to **about** 30% by weight" polyhydroxy compond, as used in Claims 19 and 20, simply cannot be interpreted to encompass a solution that contains only 4.52% by weight of a polyhydroxy compound, as is described by Ahmad et al. Thus, Applicants respectfully submit that the rejection of Claims 19 and 20 under §102(b) in view of the Ahmad et al. paper is clearly improper.

In light of the above amendment and remarks, Applicants respectfully submit that the rejection of Claims 14, 16, 17, and 19-21 Under 35 U.S.C. §102(b) in View of Ahmad et al. is untenable. Withdrawal of the same is respectfully requested.

**Rejection of Claims 14-28 Under 35 U.S.C. §102(b) in View of Roser, U.S. Patent No. 4,891,319, Issued January 2, 1990:**

This rejection is respectfully traversed because, contrary to the Office's characterization of the Roser patent, the Roser preservation solution is **devoid** of phosphate ions.

Specifically addressing Example 3 of the Roser patent, which the Office cited as being particularly relevant, the Office's attention is directed to the paragraphs at column 7, lines 5-20 of Roser. These paragraphs describe the preservation solution that Roser actually tested in Example 3. Each well contained 50 $\mu$L of the antibody to be preserved, and 50 $\mu$L of a trehalose solution containing from 0.1 % w/v to 10% w/v trehalose in distilled water plus 5

units of heparin and 0.01% sodium azide. Note that this section closes with the passage "The plates are then dried overnight at 37°C in a warm room. They can then be stored at room temperature indefinitely." Roser, column 7, lines 18-20. Roser then goes on to describe the results of the example **after** the preserved samples are re-hydrated. See Roser, column 7, lines 20-35.

One thing is immediately clear from this passage of the Roser patent: Roser's preservation solution **does not** contain any phosphate ions whatsoever. None.

In fact, the Roser patent is directed entirely and completely to the benefits of trehalose solutions as preservation agents. Phosphate is neither mentioned as an active agent, nor suggested as a beneficial ancillary ingredient. The Roser patent is simply silent regarding any type of preservation solution that contains phosphate ion **as an active agent or an ancillary agent** of the solution. To buttress the point, note Roser's Example 2, at column 6. Here, plain phosphate-buffered saline was used as a control solution.

As noted immediately above, Roser's Example 3 uses a solution that is devoid of phosphate ions as the preservation solution. Roser did, however, perform a titration in Example 3 that utilized phosphate-buffered saline (PBS). But the titration performed in Roser's Example 3, starting at column 7, line 58, was done "to establish whether there is an optimum concentration of trehalose for preservation." In no way does this titration teach or suggest that phosphate ions are to be added to Roser's preservation solution, or serve any purpose in the solution. The test was a "titration" to alter the concentration of the antibodies contained in each well. The PBS was simply used as a vehicle to serially dilute the antibody samples into the 96-well plate.

Moreover, a closer analysis of Roser's Example 3 shows that the samples described in the paragraph spanning columns 7 and 8 of Roser **do not** contain anywhere near the ratio of phosphate ions to hydroxyl groups required by the present claims. The ratio positively required by Claim 1 is from about 0.025 to about 0.625. In stark contrast, the samples described at the top of column 8 of the Roser patent have a ratio of phosphate ions to hydroxyl ions ranging from **2.44** to **244**.

The ratios in the Roser patent are calculated as follows: Roser does not provide the volume of the initial antibody samples described at the bottom of column 7. Roser does, however, state that the initial sample were "titrated 1:3 across a 96 well microtiter plate in Dulbecco's phosphate buffered saline (PBS) in a final volume of 50 μL." That means that some, if not most, of that final 50 μL volume was the PBS and some was the unnamed vehicle Roser used to prepare the initial samples (very likely the distilled water/heparin/sodium azide solution described at the top of page 7). For the calculations that follow, it has been assumed the initial samples where prepared with a negligible amount of solvent, and therefore all of the 50 μL volume was PBS.

As shown in Exhibit A, attached hereto and incorporated by reference, PBS is 0.0057 M phosphate (4.3 mM from $Na_2HPO_4$ and 1.4 mM from $KH_2PO_4$). Thus, the initial 50 μL samples described at the bottom of column 7 of Roser are 0.0057 M phosphate.

To each 50 μL sample was added another 50 μL of a trehalose solution in distilled water, thus yielding a final sample volume of 100 μL. See the very top of column 8 of the Roser patent. By doubling the total volume of the sample, without adding any additional phosphate ions, the molarity of phosphate ions present in each sample is cut in half, from 0.0057 M to 0.00285 M. It is at this point that Roser allows the solutions to air dry. Again, see the top of column 8 of the Roser patent. Thus, immediately prior to air-drying, the 100 μL samples at the top of column 8 or Roser have **a phosphate molarity of 0.00285 M**.

Each 100 μL sample also contains from 0.05 μg to 5 μg of trehalose. The calculation is as follows: At the top of column 8 of Roser, 50 μL of a solution ranging from 0.1% w/v to 10% w/v trehalose is added. A 0.1% w/v solution contains 0.001 μg trehalose/μL, while a 10% w/v solution contains 0.1 μg trehalose/μL. Thus, at the lower end of the range, the final 100 μL of solution contains from 0.05 μg trehalose (50 μL x 0.001 μg trehalose/μL) to 5 μg of trehalose (50 μL x 0.1 μg trehalose/μL).

Trehalose has a molecular weight of 342.2. Therefore, the 100 μL samples described at the top of column 8 of Roser have trehalose molarities running from 1.46 μM at the low end and 0.146 mM at the high end. The two calculations appear as follows:

(0.05 $\mu$g trehalose/100 $\mu$L) x ($10^6$ $\mu$L/L) x (1g/$10^6$ $\mu$g) x (1 mole trehalose/342.3 g) =

0.00000146 moles trehalose/L =

1.46 $\mu$M trehalose.

(5.0 $\mu$g trehalose/100 $\mu$L) x ($10^6$ $\mu$L/L) x (1g/$10^6$ $\mu$g) x (1 mole trehalose/342.3 g) =

0.000146 moles trehalose/L =

0.146 mM trehalose.

Trehalose has eight (8) hydroxyl groups. Thus, the molarity values in the two preceding paragraph are multiplied by eight (8) to yield the molarity of hydroxyl groups in the trehalose:

1.46 $\mu$M x 8 = 11.68 $\mu$M = 0.00001168 M hydroxyl.

0.146 mM x 8 = 1.168 mM = 0.001168 M hydroxyl.

Lastly, the relevant ratios are calculated: phosphate molarity (0.00285 M) divided by hydroxyl molarity:

0.00285 M phosphate ÷0.00001168 M hydroxyl = 244

0.00285 M phosphate ÷0.001168 M hydroxyl = 2.44

Note also that if the amount of trehalose added to the final 100 $\mu$L samples is doubled, (to 20% w/v), as noted at column 2, line 45 or Roser, the samples will have a phosphate to hydroxyl ratio of: **1.22**. The calculation is as follows:

A 20% w/v solution contains 0.2 $\mu$g trehalose/$\mu$L. The final 100 $\mu$L of solution contains 10 $\mu$g trehalose (50 $\mu$L x 0.2 $\mu$g trehalose/$\mu$L). The molarity of the solution is given by (10.0 $\mu$g trehalose/100 $\mu$L) x ($10^6$ $\mu$L/L) x (1g/$10^6$ $\mu$g) x (1 mole trehalose/342.3 g) = 0.000292 moles trehalose/L = 0.000292 M trehalose. This value is multiplied by eight (8) to yield the molarity of hydroxyls present in the trehalose: 0.000292 x 8 = 0.00233. The phosphate molarity has not changed, so when Roser's 20% w/v solution of trehalose is used, the final ratio is 0.00285 M phosphate ÷0.00233 M hydroxyl = 1.22.

Note that when Roser's sample contains **more** trehalose, the ratio of phosphate ions to hydroxyl groups **decreases**, from 244 when 0.01% w/v trehalose is used, to 2.44 when 10%

w/v trehalose is used, to 1.22 when 20% w/v trehalose is used. Roser, however, explicitly states that the amount of trehalose is his approach falls between 0.05% and 20% (column 2, line 45), a range that will **never** yield the phosphate to hydroxyl ratio positively required by the present claims.

Thus, the relevant phosphate to hydroxyl ratio positively required by the present claims is not taught or suggested by the Roser patent. Applicants therefore respectfully submit that the rejection of Claims 14-28 under 35 U.S.C. §102(b) in view of the Roser patent is improper. Withdrawal of the same is respectfully requested.

**Rejection of Claims 14-28 Under 35 U.S.C. §102(b) in View of Carpenter et al., U.S. Patent No. 4,806,343, Issued February 21, 1989:**

This rejection is believed to have been overcome, in part, by appropriate amendment to Claims 14 and 23, and is, in part, respectfully traversed. As applied to Claims 14-18, 21-24, 27, and 28, this rejection is believed to have been overcome by appropriate amendment to Claims 14 and 23. As applied to Claims 19, 20, 25, and 26, this rejection is respectfully traversed.

Applicants traverse this rejection, in part, because most of the Examples in the Carpenter et al. patent cited by the Office do not satisfy the positive limitations in the claims.

Specifically, Examples 1 and 5 of the Carpenter et al. patent are irrelevant to the claimed invention because the samples do not contain a sufficient amount of the polyhydroxy compound as required by the claims. Note that in Example 1, the samples contain 100 mM (=0.100 M) trehalose. See Carpenter et al., column 4, line 40. In Example 5, the samples contain 100 mM (=0.100 M) of the disaccharides sucrose, lactose or cellobiose, or the monosaccharides inositol, glycerol, sorbitol, glucose, galactose, and fructose. See Carpenter et al., column 5, lines 45-52.

A 0.100 M solution of any of the disaccharides trehalose, sucrose, lactose, and cellobiose is equal to a solution that is 3.31% by weight of the disccharide. All four of these disaccharides have the same molecular weight (342.3), so the calculation is identical for each. The calculation proceeds as follows:

0.100 moles disaccharide/liter x (342.3 g/mole disaccharide) =

34.23 g disaccharide/liter

34.23 g disaccharide/1034.23 g [*i.e.*, the weight of 1 L of the solution] x 100% =

**3.31 wt% disaccharide**.

For the monsaccharides inositol, glycerol, sorbitol, glucose, galactose, and fructose, the corresponding calculations appear as follows:

Inositol, glucose, galactose and fructose all have the same molecular weight: 180.16. Therefore:

0.100 moles monosaccharide/liter x (180.16 g/mole monosaccharide) =

18.02 g monosaccharide/liter

18.02 g monosaccharide/1018.02 g [*i.e.*, the weight of 1 L of the solution] x 100% =

**1.77 wt% inositol, glucose, galactose or fructose**.

Glycerol has a molecular weight of 92.09. Therefore:

0.100 moles gycerol/liter x (92.09 g/mole glycerol) =

9.209 g glycerol/liter

9.209 g glycerol/1009.209 g [*i.e.*, the weight of 1 L of the solution] x 100% =

**0.91 wt% glycerol**.

Sorbitol has a molecular weight of 182.17. Therefore:

0.100 moles sorbitol/liter x (182.17 g/mole glycerol) =

18.217 g sorbitol/liter

18.217 g sorbitol/1018.217 g [*i.e.*, the weight of 1 L of the solution] x 100% =

**1.79 wt% sorbitol**.

Thus, as shown by the above calculations, none of the solutions described in Examples 1 and 5 of the Carpenter et al. patent contains from 10% to about 60% by weight of a polyhydroxy compound, as is positively required by the present claims.

In Example 9 of Carpenter et al., the corresponding values also do not teach or suggest the positive values recited in the claims as amended. Specifically, Example 9 of Carpenter et al. uses a collection of solutions that are 0.06 M, 0.100 M, or 0.300 M trehalose. See Carpenter et al., column 7, lines 20-32. None of these solutions are the same as a solution

that is at least 10% by weight trehalose (or some other polyhydroxy compound), as is positively required by the present claims. The calculations appear as follows:

0.06 moles trehalose/liter x (342.3 g/mole trehalose) =
20.54 g trehalose/liter
20.54 g trehalose/1020.54 [*i.e.*, the weight of 1 L of the solution] =
**2.01 wt% trehalose**

0.100 moles trehalose/liter x (342.3 g/mole trehalose) =
34.23 g trehalose/liter
34.23 g trehalose/1034.23 g [*i.e.*, the weight of 1 L of the solution] x 100% =
**3.31 wt% trehalose**.

0.300 moles trehalose/liter x (342.3 g/mole trehalose) =
102.69 g trehalose/liter
102.69 g trehalose/1102.69 g [*i.e.*, the weight of 1 L of the solution] x 100% =
**9.31 wt% trehalose**.

The positive language of all of the present claims requires that the solution used in the method have at least 10 percent by weight of a polyhydroxy compound. Because the Carpenter et al. patent does not disclose the use of such a solution, the rejection of Claims 14-28 under 35 U.S.C. §102(b) in view of Carpenter et al. is untenable. Withdrawal of the rejection is respectfully requested.

## CONCLUSION

Applicants submit that the application is now in condition for allowance. Early notification of such action is earnestly solicited.

Respectfully submitted,

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# CURRENT PROTOCOLS IN MOLECULAR BIOLOGY

VOLUME 4





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***HeBS (HEPES-buffered saline) solution, 2×***

16.4 g NaCl
11.9 g HEPES acid
0.21 g $Na_2HPO_4$
800 ml $H_2O$
Titrate to pH 7.05 with 5 M NaOH
Add $H_2O$ to 1 liter
Filter sterilize through a 0.45-μm nitrocellulose filter
Test for transfection efficiency and store at –20°C in 50-ml aliquots

*An exact pH is extremely important for efficient transfection. The optimal pH range is 7.05 to 7.12.*

***KCl, 1 M***

74.6 g KCl
$H_2O$ to 1 liter

***$MgCl_2$, 1 M***

20.3 g $MgCl_2 \cdot 6H_2O$
$H_2O$ to 100 ml

***$MgSO_4$, 1 M***

24.6 g $MgSO_4 \cdot 7H_2O$
$H_2O$ to 100 ml

***MOPS buffer***

0.2 M MOPS [3-(*N*-morpholino)propanesulfonic acid], pH 7.0
0.5 M sodium acetate
0.01 M EDTA

*Store in the dark and discard if it turns yellow.*

***NaCl, 5 M***

292 g NaCl
$H_2O$ to 1 liter

***NaOH, 10 M***

Dissolve 400 g NaOH in 450 ml $H_2O$
Add $H_2O$ to 1 liter

***PBS (phosphate-buffered saline)***

| *10× stock solution, 1 liter:* | *Working solution, pH ~7.3:* |
|---|---|
| 80 g NaCl | 137 mM NaCl |
| 2 g KCl | 2.7 mM KCl |
| 11.5 g $Na_2HPO_4 \cdot 7H_2O$ | 4.3 mM $Na_2HPO_4 \cdot 7H_2O$ |
| 2 g $KH_2PO_4$ | 1.4 mM $KH_2PO_4$ |

***Potassium acetate buffer, 0.1 M***

*Solution A:* 11.55 ml glacial acetic acid/liter (0.2 M).

*Solution B:* 19.6 g potassium acetate ($KC_2H_3O_2$)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with $H_2O$ to 100 ml.

*This may be made as a 5- or 10-fold concentrate by scaling up the amount of potassium acetate in the same volume. Acetate buffers show concentration-dependent pH changes, so check concentrate pH by diluting an aliquot to the final concentration.*

*To prepare buffers with pH intermediate between the points listed in Table A.2.2, prepare closest higher pH, then titrate with solution A.*